Dr. Geoffrey Hale – Chief Executive Officer of mAbsolve Ltd., UK

Therapeutic Antibodies

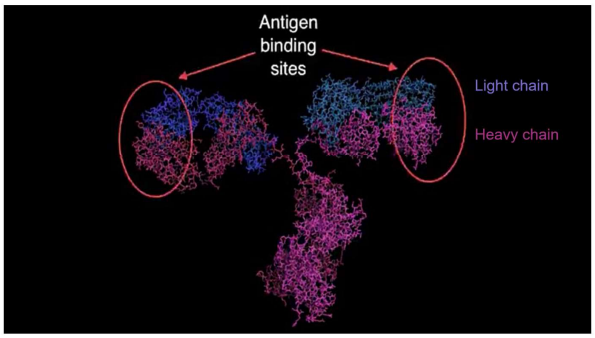
Dr. Geoff Hale

Chief Executive Officer of mAbsolve Ltd.

Formerly Professor of Therapeutic Immunology
Sir William Dunn School of Pathology
University of Oxford, UK

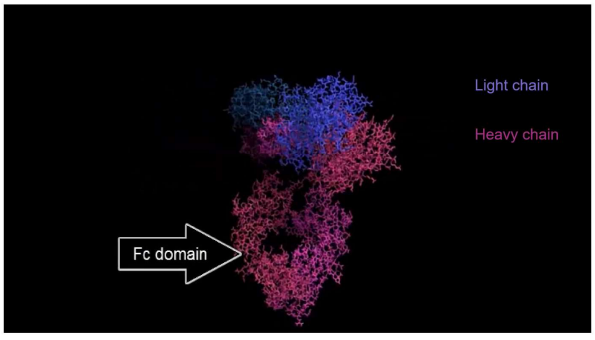
1

Antibody structure



2

Antibody structure



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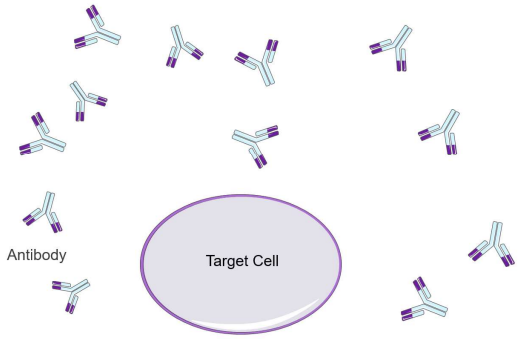
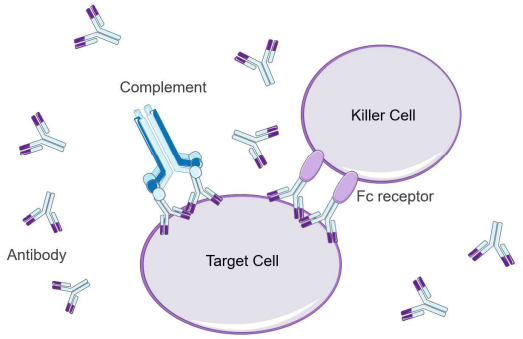
Antibodies – Nature's pro-drugs



3

Antibodies – Nature's pro-drugs



Key properties of antibodies relevant to therapy

Binding function

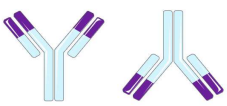
- Exquisite specificity
- High avidity from two binding sites

Long half life

- Liver FcRn receptor
- Carrier function for other drugs

Effector function

- Complement
- Fc receptors



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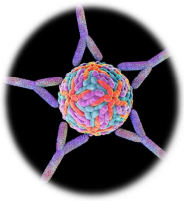
Antibodies are different from conventional drugs

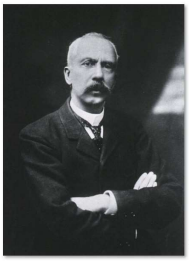
- Large Size
  - Restricted tissue distribution
- Common structure
  - Generic methodology
- Potentially immunogenic
  - Anti-globulin responses



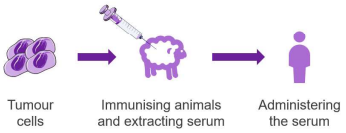
5

Treatment of a case of sarcoma by serotherapy

Héricourt J, and Richet C. Traitement d'un cas de sarcome par la sérothérapie. *C R Hebd Seances Acad Sci* 1895; **120**:948-50



Charles R. Richet



6

Conclusions of Hericourt and Richet

- Antibody therapy can ameliorate symptoms and give significant remission from disease
- It is unlikely to cure advanced disease
- In combination with other radical treatment, *e.g.* surgery, it may be a very effective adjuvant therapy for eliminating metastatic disease, possible leading to complete cure

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Paul Ehrlich 1854-1915

"You see we must take aim - aim by chemical variation! The marvellous effect of an antibody in the serum is due to the fact that in no case it has affinity for the body substances but flies straight onward without deviation, upon the parasites.

Paul Ehrlich

The antibodies are therefore **MAGIC BULLETS** which find the targets themselves…

… we must therefore concentrate all our powers and abilities on making the aim as accurate as we can contrive, so as to strike the parasites as hard and the body cells as lightly as possible."

circa 1904

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Modes of antibody therapy

Passive

- Infectious diseases: diphtheria, measles, rubella, hepatitis, tetanus, rabies, Spanish flu and Covid-19
- Anti-venoms

Active

- Cancer
- Cardiovascular disease
- Autoimmune disease and transplantation
- Anti-Rhesus

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Problems with polyclonal antisera

1 Lack of Reproducibility
- Each batch could be different

2 Complex Mixture
- Many different specificities
- Potential contaminants (*e.g.* viruses)

3 Immunogenicity
- Animal proteins provoke immune response
- Loss of efficacy and serum sickness

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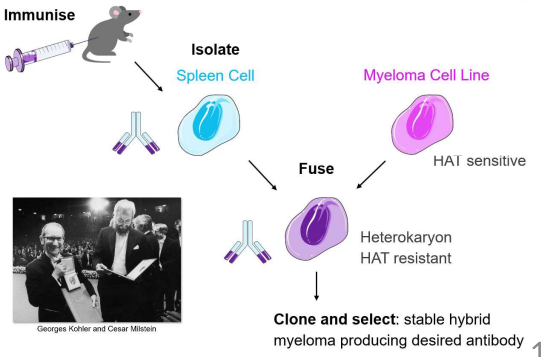
Making monoclonal antibodies



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Pros and cons of monoclonal antibodies

Unlimited supply

So long as the cell line is stable

Immunogenicity

Mouse or rat = immunogenic

Human monoclonals?

Purity

Single specificity = small doses

Variable Potency

Maybe less active than polyclonal

Selection, cocktails and modifications

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Antibodies for cell depletion

- Natural mechanisms (naked antibody)
  - Complement
  - Antibody-Dependent Cell-mediated Cytotoxicity (ADCC)
  - **Cytotoxic T cells**
- Artificial mechanisms (as carrier)
  - Conventional drugs
  - Immune activators
  - Radioisotopes
  - Toxins
  - Enzyme/pro-drug
  - Viruses, genes, *etc.*

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Genetic engineering of antibodies

- Reduce immunogenicity
  - Chimeric, humanised, phage, transgenic
- Modify effector function
  - Aglycosyl, non-FcR binding
- Create fragments or complexes
  - Hybrids, bispecific
- Improve stability
  - Human IgG4

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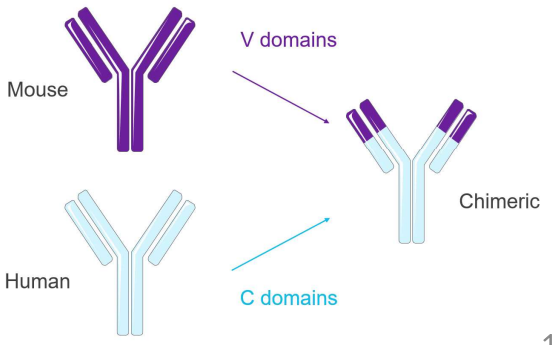
Chimeric antibodies



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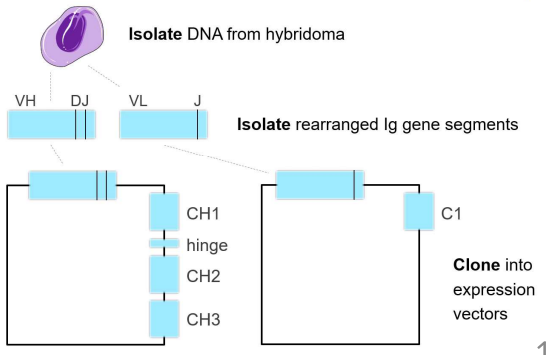
Making chimeric antibodies



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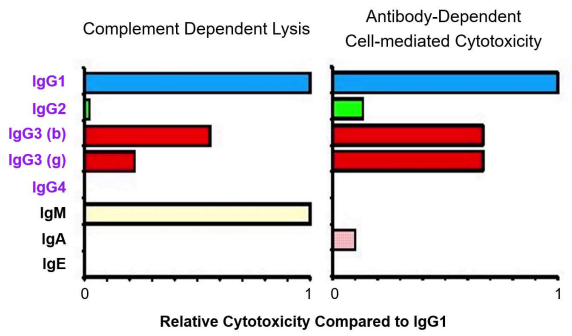
Effector functions of anti-NP antibodies



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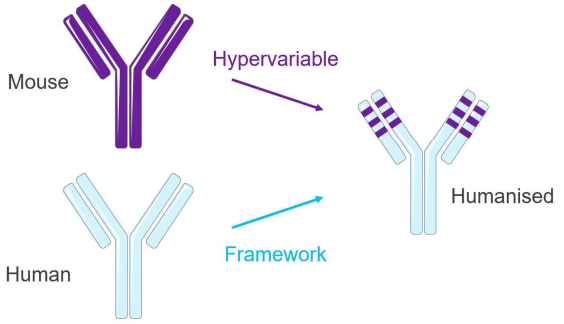
Humanised antibodies



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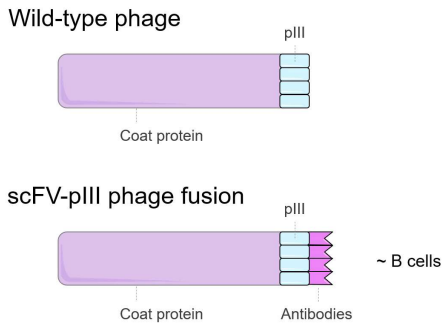
Making humanised antibodies by phage display



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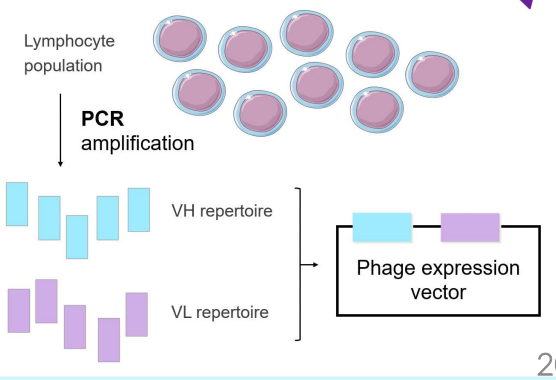
Making phage antibodies



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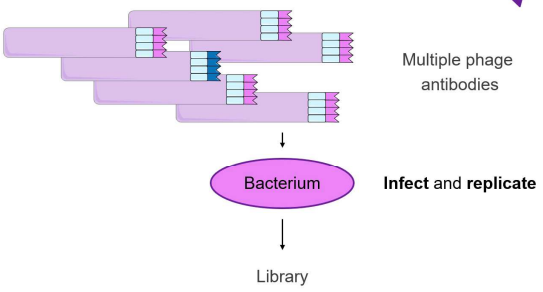
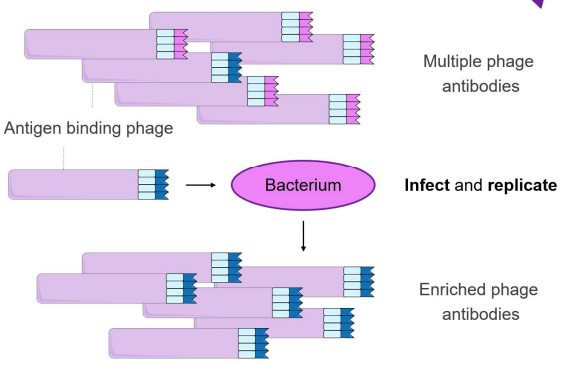
Infection, replication and enrichment



21

Infection, replication and enrichment



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Pros and cons of phage antibodies

Start from human genes New specificities? Less immunogenic?	
Can screen a larger repertoire	Loss of combinatorial specificities
Bypass immunisation – naive library	Low affinity
Affinity maturation *in vitro*	Extra steps, more immunogenic?
Economical production in bacteria	Only small fragments

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Antibodies from transgenic mice

STEP 1: Disrupt mouse mu and kappa loci
- Ignore mouse lambda

STEP 2: Introduce human mu and kappa

STEP 3: Introduce most abundant human V genes
- 50% of repertoire from 7/87 genes

STEP 4: Introduce human gamma constant regions as required
- IgG1 or IgG3

➡ **Can be immunise, get affinity maturation and class switching**

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Antibody nomenclature: source

MOUSE
…omab
Example: ibritumomab (Zevalin)

CHIMERIC
…ximab
Example: rituximab (Rituxan)

HUMANIZED
…zumab
Example: alemtuzumab (Campath)

HUMAN
…umab
Example: adalimumab (Humira)

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Immunogenicity of therapeutic Mabs

- 72 marketed therapeutic antibodies
- Incidence of anti-drug antibodies (from manufacturer's summary of product characteristics)
- Compare V-region sequences with human germ-line genes to find closest match
- Calculate likelihood of peptides binding to MHC Class 2
  - Count the number of peptides ranking in the top 1%

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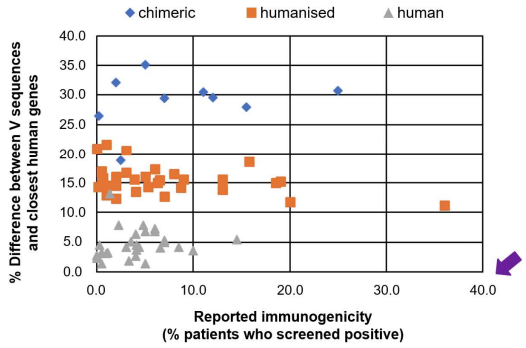
Immunogenicity



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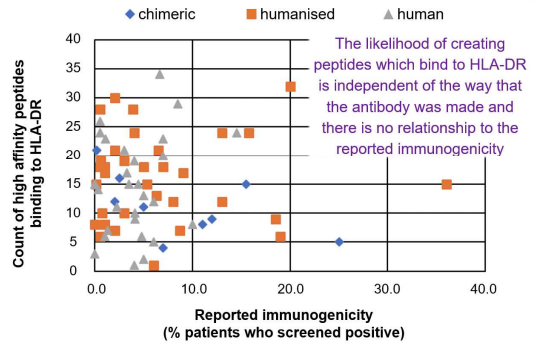
Immunogenicity



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Factors influencing the antiglobulin response

- Dose of antibody
- Number of injections
- Route of injection
- Immunocompetence of the recipient
  e.g. people with cancer or autoimmune disease
- Danger signals

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Some observations

- Chimeric, humanised or 'fully human' antibodies are less immunogenic than murine
- No published studies show difference in response between them
- Relative merits of the technologies depend on technical factors or intellectual property rights

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Increasing antibody affinity

Advantages

- **High affinity** good for diagnostics
- **Increased potency** = lower dose, fewer side effects and reduced cost?

Drawbacks

- **Cross-reactivity increased** = more side effects
- Poorer tissue penetration
- Potential for dose reduction limited by size of antigen sink

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Antibodies for tumour therapy

- Cell Depletion
  - Rituximab (naked)
  - Gemtuzumab (drug)
  - Ibritumomab (radioisotope)
- Blocking receptors
  - Trastuzumab
- Attacking vasculature
  - Bevacizumab
- **Stimulating immune response**
  - Ipilimumab

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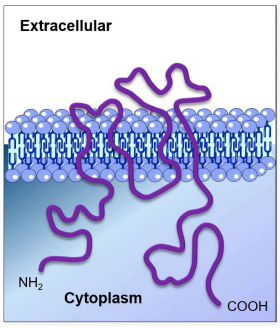
CD20 antigen



- B cell marker, discovered 1980
- Function not known, possible ion channel
- Good target for therapeutic antibodies
  - Non-modulating
  - Epitope close to membrane
  - Multimeric

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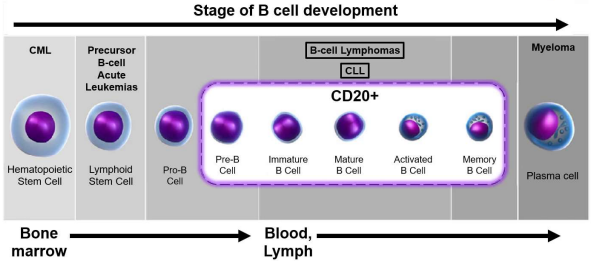
CD20 and B cell development



➡ The advantage of using an antibody which recognises such a wide range of cells is that it can be used for treating a number of different types of lymphoma

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Van Meerten and Hagenbeek., *Semin Hematol.* 2010;**47**(2):199-210

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Phase III trial of rituximab in follicular lymphoma

Clinical Trial > Blood. 2005 Feb 15;105(4):1417-23. doi: 10.1182/blood-2004-08-3175. Epub 2004 Oct 19.

CVP chemotherapy plus rituximab compared with CVP as first-line treatment for advanced follicular lymphoma

Robert Marcus [1], Kevin Imrie, Andrew Belch, David Cunningham, Eduardo Flores, John Catalano, Philippe Solal-Celigny, Fritz Offner, Jan Walewski, João Raposo, Andrew Jack, Paul Smith

- Compared combination chemotherapy (cyclophosphamide + vincristine + prednisone) with the same combination plus rituximab
- Median duration of response:
  - **CVP** ..... 14 months
  - **CVP + rituximab** ..... 35 months

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CD20 epitopes

Type I (*e.g.* Rituximab)

- Induce redistribution to rafts
- Activate ADCC and complement
- May induce 'open' configuration of Ca^{2+} channel

Type II (*e.g.* Obinutuzumab)

- No redistribution
- Activate ADCC but not complement
- Induce homotypic adhesion and cell death
- 'Closed' configuration of Ca^{2+} channel

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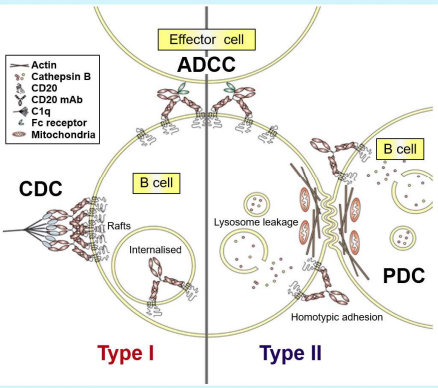
Type I and II antibodies



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Anti-CD20 antibodies

Type I

- Rituximab (MabThera, Rituxan)
  - Biosimilars
- Ofatumumab (Arzerra)
- Ocrelizumab (Ocrevus)

Type II

- Obinutuzumab (Gazyvaro, Gazyva)

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Antibodies for immunomodulation

Cell Depletion

Example: Alemtuzumab (anti-CD52)

Blocking receptors

Example: Daclizumab (anti-CD25)

Blocking cytokines

Example: Infliximab (anti-TNF)

Blocking mediators

Example: Eculizumab (anti-complement C5)

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Tumour necrosis factor (TNF)

- Coley's toxin - 1890s, stimulation of immune system with bacteria for treatment of tumours
- TNF identified in 1975
- Cytokine involved in systemic inflammation
- Secreted by macrophages, and many other cell types
- Induces fever, apoptosis, sepsis, inhibits tumorigenesis and viral replication
- Tasonermin has limited use for tumour therapy
- Anti-TNF antibodies developed for treatment of septic shock

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Physiology of TNF

- Transmembrane homotrimer is cleaved to give soluble TNF, also a trimer
- Two receptors, TNF-R1 (p55/60) and TNF-R2 (p75/80)
- Binding to receptor causes trimerisation, conformational change and results in a complex cascade of intracellular events including:
  - Activation of NF-kB and MAP kinase, and induction of death signalling
- Promotes the inflammatory response involved in a range of autoimmune diseases
  - *e.g.* rheumatoid arthritis, inflammatory bowel disease or psoriasis

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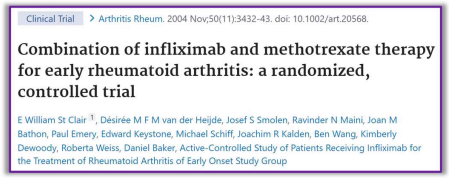
Phase III trial of infliximab in rheumatoid arthritis



Clinical Trial > Arthritis Rheum. 2004 Nov;50(11):3432-43. doi: 10.1002/art.20568.

Combination of infliximab and methotrexate therapy for early rheumatoid arthritis: a randomized, controlled trial

E William St Clair [1], Désirée M F M van der Heijde, Josef S Smolen, Ravinder N Maini, Joan M Bathon, Paul Emery, Edward Keystone, Michael Schiff, Joachim R Kalden, Ben Wang, Kimberly Dewoody, Roberta Weiss, Daniel Baker, Active-Controlled Study of Patients Receiving Infliximab for the Treatment of Rheumatoid Arthritis of Early Onset Study Group

- Compared methotrexate with methotrexate plus infliximab
- Median improvement in clinical symptoms (ACR score) at 12 months:
  - **Methotrexate** ····→ 26%
  - **Methotrexate + Infliximab** ····→ 47%

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Anti-TNF antibodies

- **Etanercept** (Enbrel)
  - Fusion protein
  - Biosimilars
- **Infliximab** (Remicade)
  - Biosimilars
- **Adalimumab** (Humira)
  - Biosimilars
- **Certolizumab pegol** (Cimzia)
- **Golimumab** (Simponi)

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CD52 antigen

Expressed on:
Approx. 5% of lymphocyte surface

CD52 antigen

Exceptionally lytic with human complement

Different isotypes and variants:

- Rat IgM, rat IgG2a and rat IgG2b
- Humanised IgG1 (alemtuzumab)

Clinical applications:

- Leukemia and lymphoma
- Autoimmune diseases
- Bone marrow and organ transplantation

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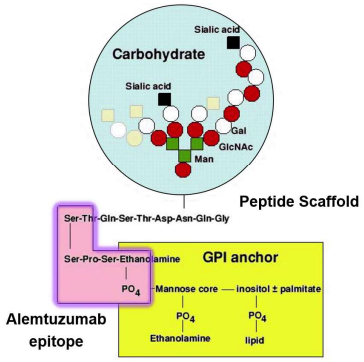
CD52 antigen structure



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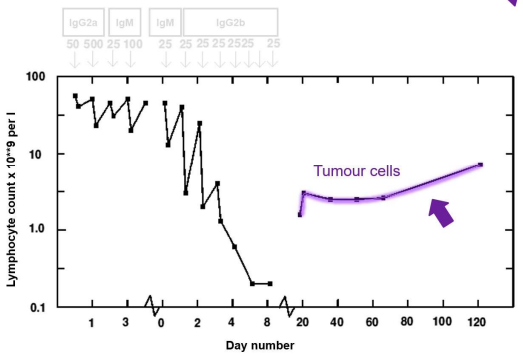
Treatment of leukaemia with rat CD52 antibodies



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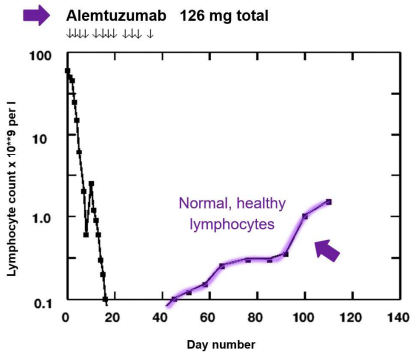
Treatment of lymphoma with alemtuzumab



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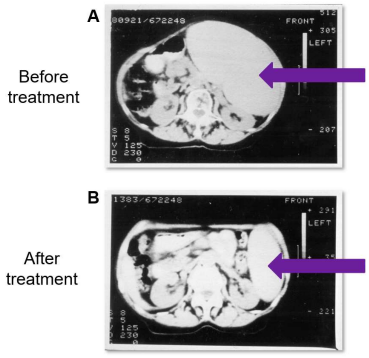
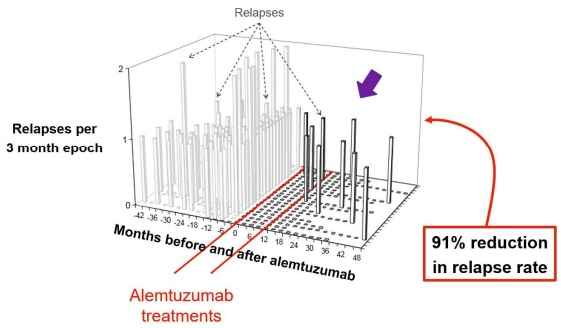
Treatment of lymphoma with alemtuzumab



Alemtuzumab effectively suppresses relapses



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Coles AJ., *J. Neurol.* 2006; **253**: 98-108

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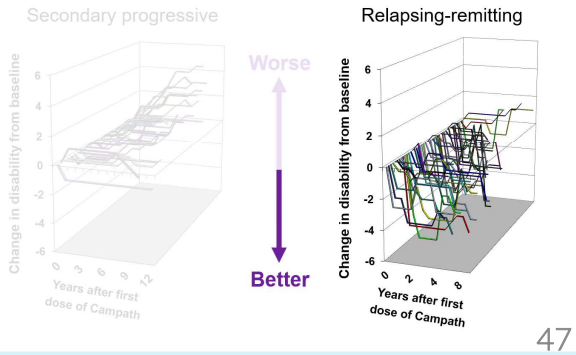
Change in disability after alemtuzumab treatment



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Phase III trial of alemtuzumab in multiple sclerosis

Clinical Trial > Lancet. 2012 Nov 24;380(9856):1819-28. doi: 10.1016/S0140-6736(12)61769-3. Epub 2012 Nov 1.

Alemtuzumab versus interferon beta 1a as first-line treatment for patients with relapsing-remitting multiple sclerosis: a randomised controlled phase 3 trial

Jeffrey A Cohen [*], Alasdair J Coles, Douglas L Arnold, Christian Confavreux, Edward J Fox, Hans-Peter Hartung, Eva Havrdova, Krzysztof W Selmaj, Howard L Weiner, Elizabeth Fisher, Vesna V Brinar, Gavin Giovannoni, Miroslav Stojanovic, Bella I Ertik, Stephen L Lake, David H Margolin, Michael A Panzara, D Alastair S Compston, CARE-MS I Investigators

- Compared beta-interferon with alemtuzumab
- Freedom from relapse at 2 year:
  - **Beta-interferone** ····· 59%
  - **Alemtuzumab** ····· 78%

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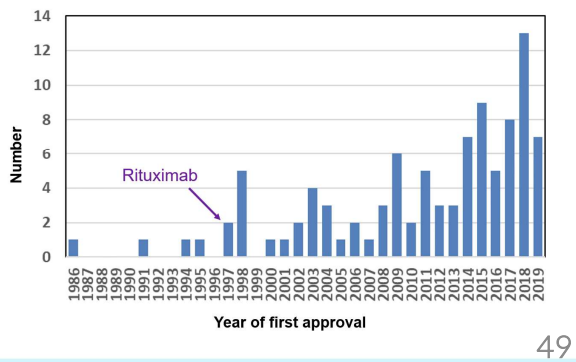
Approval of antibodies and fusion proteins



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HSTalks

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Therapeutic antibodies & fusion proteins

- **2019:** 97 antibodies, 65 antigens
- 6 mouse, 1 mouse/rat, 9 chimeric, 42 humanized, 32 human, 7 Fc fusion
- IgM, IgG1, IgG2, IgG4, Fab, bispecific, scFv
- Transplant rejection, heart disease, cancer, autoimmunity, infection, allergy, genetic disorders, macular degeneration, hypercholesterolemia, drug overdose, migraine, *etc.*

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Thank you!

- In past years my research on therapeutic antibodies was supported by various grants from government and pharmaceutical companies. I also received royalties from the sales of antibodies
- Today I am a founder and director of a number of small biotech companies. One of them, Absolute Antibody, works to engineer antibodies for research, diagnosis and therapy
- I hope that you found something of interest here, and I would love to meet you one day to hear about your work

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See related information in the links tab

HSTalks

By leading world experts